ON GOLD AS A STAINING AGENT FOR NERVE TISSUE.

By Dr. HENRY S. UPSON,

OF CLEVELAND, O.

(*Second Paper.*)

IN staining nerve structures with chloride of gold, as was pointed out in the previous paper on this subject, the differentiation takes place mainly during the process of hardening, so that it is necessary that this should be conducted with extreme care. At the same time, the treatment with the reducing fluid is in a certain sense the pivotal process, as it is at this time that the section takes the actual stain.

With the process given in that paper, very little depends on the way of manipulating the sodium hydrate solution or the reducing fluid; I believe that with ordinary care the stain obtained varies accurately with the differences in the tissue and in the hardening process. The factors of which we must take account in hardening are: First, the length of time which elapses between death and the bringing of the tissues into Müller's fluid. Second, the temperature at which the process is conducted. Third, the length of time in Müller's fluid. Fourth, the length of time in each of the successive reagents during the imbedding and cutting process, and, after this, before staining.

It has long been an object with the profession to find a stain or, at any rate, a chemical test capable of showing changes in the tissues too subtle to result in a change in the form of the elements. If, as seems probable, some or all functional diseases are the expression of molecular or chemical variations from the normal, this is an advance which may reasonably be expected. It is evident that a method delicate enough to accomplish this purpose must also show such marked changes as occur in the body within a moderate time after death.

From my observations on gold staining thus far, I think that post-mortem changes are shown by an increased liability of the myeline sheaths and neuroglia to take the stain, making such changed tissues harder to differentiate.

If in pathological specimens we can eliminate this source of error by bringing the tissues fresh into Müller's fluid, if they are then hardened at a fixed temperature and under identical conditions with normal tissue, then any greater tendency on the part of the myeline sheaths of the tissue in question to take the stain will be evidence of ante-mortem change.

It is often impossible to obtain pathological specimens until after post-mortem changes have begun. It may then be necessary, before sections are brought into the gold chloride solution, to bring them for a day or two into a four per cent. alcoholic solution of sulphuric acid.

The following method will be found better in most respects than the preceding one:

Method No. 2.—To 30 c. c. of a 1 per cent. tincture of iodine add 1 gramme of proto-chloride of tin, and label this tin solution.

Make a 5 per cent. solution of phosphate of iron, and label this iron solution; the scale salt should be used, as prepared for medicinal purposes.

Tissues should be hardened in Müller's fluid and in alcohol, and the sections cut and preserved, as in the preceding method.

The section to be stained is brought for half an hour or more into a 1 per cent. solution of gold chloride; it is then washed for a moment in water, and brought for half a minute or more into a 10 per cent. solution of sodium hydrate, to which has been added one-half per cent. or less of ammonium vanadate; it is then washed in water and brought into the reducing fluid freshly made as follows:

Tin solution, ...	gtt. xv.
Distilled water, ...	c. c. iii.
Iron solution, ...	gtt. iii.
Sulphurous acid, ...	c. c. iii.

The section in a few moments takes a somewhat metallic purple color; it is then washed in water and mounted in Canada balsam as usual.

Ganglion cells and axis cylinders are sharply stained, and the endothelium of the smaller blood-vessels is apt to be stained, showing the vessels in outline; myeline sheaths, connective-tissue nuclei, and glia tissue are for the most part unstained; the glia tissue in the posterior horns and around the central canal is most apt to be colored.

This method may be varied almost indefinitely.

Chromic acid may be added to the sodium hydrate solution in place of ammonium vanadate. A mere trace will be sufficient. The stain is then more reddish in color.

Iodide of tin may be bought as such, or made by adding either tincture of iodine or potassium iodide to tin protochloride.

Ferrous sulphate or ferric nitrate may be used instead of the phosphate of iron.

Nitrate of tin gives a remarkably clear picture of the ganglion cells, but is difficult to obtain.

This method requires comparatively little oxide of chromium in the tissues, and gives a clear stain in sections after a much longer stay in alcohol than is possible with the first method.

If the tissues still contain too much of the chrome salt, sections should be brought for a day or two into a 4 per cent. solution of sulphuric acid before staining.

The above methods depend on two principles:

First.—The reduction of metallic salts; this may happen by the abstraction of the metal or metallic oxide by the tissue, oxygen being set free, or by the abstraction of oxygen by a reducing agent, leaving a lower oxide of the metal combined with the tissues. Thus, in hardening, potassium bichromate ($K_2CrD_4CrO_3$) is in solution; it is decomposed, chromium trioxide (CrO_3) is taken up by the tissues either in this form or as chromium dioxide (CrO_2), which gives to the tissues a brown color; later, more oxygen is lost, leaving chromium sesquioxide (Cr_2O_3), which gives to the tissues a green color; potassium chromate

(K_2CrO_4) remains in solution and gives to the hardening fluid a brown color instead of the original yellow.

Second.—The substitution of one metallic salt for another in an insoluble compound. The chromic salt, either the dioxide or the sesquioxide, is replaced by the gold salt, which is then reduced by further manipulation.

Gold replaces silver in a similar way, with this difference: in a section stained by the Golgi or other silver method and brought into gold chloride solution, the purple oxide of gold is deposited at once, following accurately the lines of the silver salt. In this way the stain is rendered sharper and more durable. If now a silver specimen treated in this way, instead of being mounted at once, is brought successively into sodium hydrate solution, and either potassium iodide solution or the reducing fluid given in the previous paper, the unstained parts take a bright red color, thus giving a double gold stain; the original silver stain is in purple, everything else in red. This, with some other observed facts, tends to show that in hardening with Müller's fluid all tissues are impregnated with chrome salts, and that these are replaced throughout by gold or silver salts, as the case may be. The differentiation results from an unequal reduction due largely to a difference in the form in which the chrome salts are deposited in the different tissues.

The purple color resulting from the treatment of gold chloride with a tin salt is called the purple of Cassius. In this case it is probable that the oxide of vanadium and the oxide of chromium respectively enter into the formation of the color; whether there is also present any oxide of tin is very doubtful.